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A SURVEY OF THE CHOLINE CONTENT OF GRAINS, HAYS and BY-PRODUCTS USED IN ANIMAL and POULTRY FEEDS.

B y

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April 13th, 1946.

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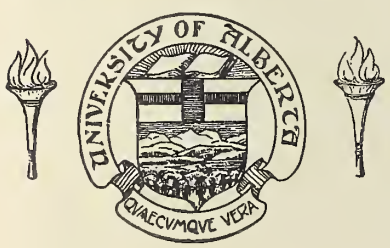

A Survey of the Choline Content of Grains, Hays and By-Products Used in Animal and Poultry Feeds.

By Hugh Alexander Rigney.

A Thesis

submitted to the Department of Animal Science of the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science.

University of Alberta,

April 13th, 1946.

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A SURVEY OF THE CHOLINE CONTENT OF GRAINS, HAYS AND BY-PRODUCTS USED IN ANIMAL AND POULTRY FEEDS.

INTRODUCTION.

Choline is generally considered to be a member of the vitamin B complex and is recognized as an important nutritional factor. It is one of the factors required by poultry for the prevention of perosis, and has been found to be a dietary factor involved in the prevention of fatty livers and certain kidney disorders in small experimental animals. Fatty liver in feed lot cattle and sheep has become a problem in Alberta, but no experimental work has been done to determine whether choline deficiency is a factor in this syndrome.

An essential preliminary step to animal experimentation on this problem is a knowledge of the choline content of livestock feeds. Data on the choline content of poultry feeds would be of immediate practical value in estimating to what extent poultry rations may require supplementation with this factor. The choline content of feedstuffs can at present be gauged only from very meager data available for feeds grown under conditions of soil and climate quite different from those prevailing in western Canada. A survey of the choline content of

grains, hays and by-products used in animal and poultry feeds was undertaken with these considerations in mind.

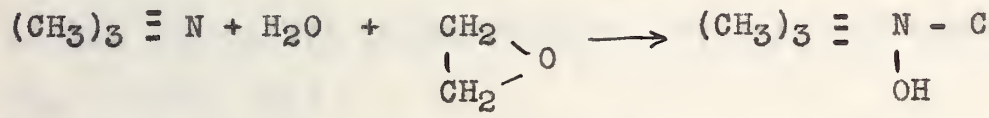
Crop samples collected for another research project in progress at the University of Alberta under the title "Soil and Plant Relations Affecting the Nutritional Value of Foods and Feeds" were available and constituted excellent material for a survey of the choline content of farm grown Alberta feeds. By-products used in livestock and poultry feeds were collected from milling companies, dairies, packing plants and feed manufacturers.

The methods used to assay these feeds for choline and the results obtained are described in this thesis. A brief review of literature precedes the presentation and interpretation of the experimental section.

REVIEW OF LITERATURE.

Chemistry.

Choline is trimethyl hydroxyethyl ammonium hydroxide, a base of comparable strength to sodium hydroxide. It is produced synthetically from trimethyl amine and ethylene oxide according to the equation:



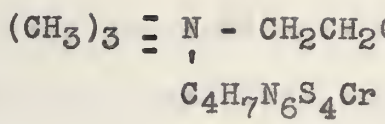
Trimethyl amine Ethylene Oxide Choline

Choline is a colorless, viscid, liquid, very hygroscopic and absorbs carbon dioxide from the air. It is odorless, has a caustic bitter taste, and is readily decomposed at elevated temperatures to give off trimethylamine and glycol. It is soluble in methyl and ethyl alcohol, but insoluble in ether and many other solvents.

Choline is available commercially chiefly as the chloride. Choline chloride is hygroscopic and very soluble in water and alcohol; aqueous solutions are practically neutral.

The reineckate salt is used in chemical assay work. Properties which make this possible are that this salt is very insoluble in water, quite insoluble in absolute ethyl alcohol, and n-propanol and the fact that it forms a colored solution in acetone. The importance of these features will be noted in the section on assay procedure.

Formula:

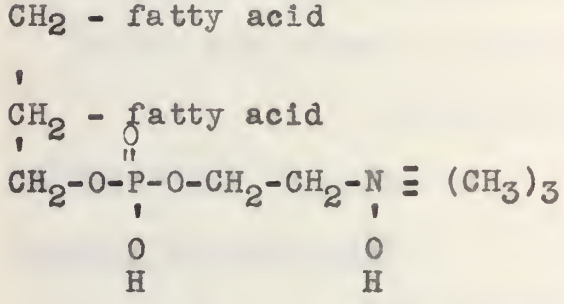


$(CH_3)_3 \equiv N - CH_2CH_2OH$

$C_4H_7N_6S_4Cr$

Choline reineckate.

Choline does not occur free in nature to any great extent, but rather in combined form with two of the recognized phospholipids, lecithin and sphingomyelin.

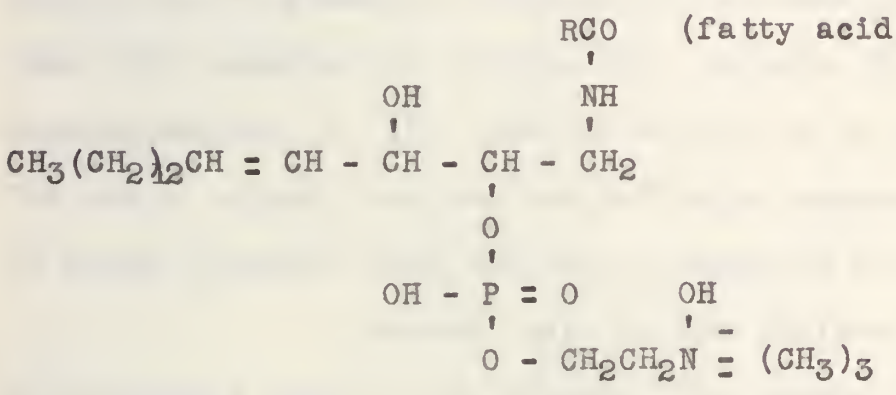


CH_2 - fatty acid

CH_2 - fatty acid

$CH_2-O-P(=O)(OH)-O-CH_2-CH_2-N^{+} \equiv (CH_3)_3$

lecithin

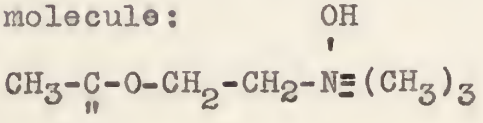


$CH_3(CH_2)_{12}CH = CH - CH(OH) - CH(NH(RCO)) - CH_2O$

$P(=O)(OH) - O - CH_2CH_2N^{+} \equiv (CH_3)_3$

sphingomyelin

It also occurs as part of the acetylcholine molecule:



$CH_3-C(=O)-O-CH_2-CH_2-N^{+}(OH) \equiv (CH_3)_3$

acetyl choline

Physiological Action.

The physiological functions of choline have been reviewed by the author in another paper (1) and may be summarized briefly as follows:

1. Choline stimulates the transport of fat throughout the body, and especially from the liver to the depots.
2. It is important in the formation of the neurohormone acetylcholine.
3. It takes part in certain transmethylation processes which are known to occur in the living organism.

The physiological importance of choline is therefore evident.

Dietary Requirements.

Experimental evidence indicates that choline is an essential metabolite for rats (2), mice (3), dogs (4), rabbits (5), chicks (6), turkeys (7), and certain microorganisms (8, 9). Whether or not it is essential for other animals and man has yet to be demonstrated, but it seems probable that choline is required by most species.

Precise data on the choline requirement of different species are meager. The levels suggested to date are summarized in Table 1.

Table 1.

Dietary Choline Required by Different Species.

Animal	Suggested Choline* Requirement
Depancreatized dog	35 mg./kg. daily (10)
Intact young dog	50 mg./kg. daily (4)
Young rats	4-6 mg. daily (2)
Young rabbits	300 mg. daily (5)
Young turkeys (1st 4 weeks)	0.2% of diet (6)
Young chicks (1st 4 weeks)	0.1% of diet (7)
Neurospora crassa - Mutant	20 micrograms per flask gives maximum growth (9)

Assay Methods.

When the nutritional importance of choline came under study, the need for a rapid, simple and accurate method of assay became apparent. Three methods of assay have been used:

1. Biological
2. Microbiological
3. Chemical
   (a) Gravimetric
   (b) Colorimetric

The biological method is slow and expensive, and has a number of other disadvantages which have prevented its widespread use. Such factors as tissue breakdown, which results in the liberation of choline from the body phospholipids, "labile methyl" groups in the feed and other factors which may increase or decrease the choline requirement are not completely understood at present.

* Expressed as choline chloride.

Engel (11) has proposed an assay procedure based on the survival of weanling rats, but this method is not in common use since methionine interferes with the assay. Jukes (15) has reported a method of rating foods for their antiperotic activity in chicks, but here again certain nutritional conditions, especially the feeding of corn, appear to have an aggravating effect on the incidence of perosis and until the conditions are more fully understood, the perosis prevention method is of limited application.

To date microbiological methods of choline assay have received only preliminary study, but in the light of the proven speed and specificity of microbiological assays for other essential nutrients, it seems certain that this field will be more thoroughly explored in the future. Horowitz and Beadle (9) have described a microbiological assay using a cholineless mutant of the mold Neurospora crassa. This mold is at present being quite widely used to test biological fluids such as blood, urine, etc., in which minute quantities of free choline are found. Total choline may also be assayed by this organism if the sample is first hydrolysed to free the combined choline.

When the knowledge of interfering substances is more complete, the accuracy of biological and microbiological assays for choline is certain to improve, but at present chemical methods offer the most promise with respect to speed and accuracy.

The most accurate chemical methods in use today are based on the precipitation of choline with Reinecke salt to form choline reineckate, which can be dissolved and measured colorimetrically. The most popular method is Glick's (12) modification of the procedure described by Engel in 1942 (11). In brief, the method consists of extracting the material with absolute methanol, reducing the extract to near dryness, hydrolysing the residue with boiling aqueous barium hydroxide, neutralizing with acetic acid and finally precipitating the choline at 5°C. by the addition of Reinecke salt. The choline assays reported in this thesis were done using this method.

Concentration and Distribution of Choline in Foodstuffs.

Information on the distribution of choline in foodstuffs is limited, and the values given are general. Fletcher et al (13), Rhian et al (14) and Jukes (15) have by biological methods evaluated a number of feeds for choline. Rhian et al (14), Engel (16) and Glick (17) assayed a number of feeds by chemical methods.

It has been found that among animal products the richest source of choline is egg yolk, while visceral organs, heart and nerve tissue, are much better sources than muscle tissue. The choline content of animal materials is directly related to their phospholipid content. It has been reported that fish meal is a better source of choline than is tankage, and that tankage is a somewhat better source than meat meal.

In cereal grains the choline is concentrated in the germ and outer coats. In corn up to 75% and in wheat about 50% of the choline is found in the germ and outer coats, thus wheat bran and shorts are substantially better sources of choline than is whole wheat.

In almost all cases the reported values represent only a few samples, often only one. It is impossible to draw conclusions from such a limited number of assays. The choline content of plant products in particular may be highly variable, since there is some evidence that it may be influenced by variations in soil and climate, etc. For example, Engel (16) reported a value of 1.39 mg. of choline chloride per gram of barley, while Glick (17) using barley samples from different locations reported 1.10 mg./gm. as the average for seven samples, with a range of 0.96 to 1.20 mg./gm. On the basis of a reasonably large number of assays Glick (17) has shown that choline values may vary not only in different kinds of grain, but in different varieties of the same grain in different localities. No conclusion was possible as to whether variety or environment was the most important factor governing variation.

EXPERIMENTAL.

Assay Materials.

It has already been mentioned that crop samples collected for another research project in progress at the University of Alberta under the title "Soil and Plant Relations Affecting the Nutritional Value of Foods and Feeds" were available and constituted excellent material for a survey of the choline content of farm grown Alberta feeds. These samples had been chosen as representative samples from the different soil zones of Alberta.

Other materials such as plant and animal by-products and a few grains and grasses commonly used in livestock and poultry feeds were procured from various processors and feed manufacturers.

Grain and grass samples were finely ground in a Wiley mill before assay. Most of the by-products studied were finely divided when received, and were assayed without further preparation.

Assay Procedure.

Duplicate air-dry samples estimated to contain the equivalent of 4 to 8 mg. of choline chloride per sample were weighed and placed in 22X88 mm. alundum extraction thimbles of medium porosity. The thimbles were loosely plugged with cotton. Since the apparatus used would not accommodate larger extraction thimbles, it was necessary in the assay of some bulky, low choline feeds

such as hay and straw to use samples containing less than the equivalent of 4 mg. of choline chloride.

In accordance with the suggestion of Glick (12), #2 pumice was added to finely divided materials to facilitate extraction by preventing the formation of hard cakes. The samples with which pumice was used and the amount employed in each case are listed in Appendix Table I.

The thimbles were placed in Soxhlet apparatus fitted with 125 ml. boiling flasks containing approximately 100 ml. of redistilled C.P. methanol. A boiling chip was added to prevent bumping and extraction was allowed to proceed for 24 hours on a six unit crude fibre extraction apparatus fitted to handle Soxhlets. Three duplicate samples could be run at one time on this unit.

At the end of the extraction the boiling flasks were placed on a steam bath and the methanol was evaporated to a volume of 4 to 6 milliliters. Thirty milliliters of a saturated solution of barium hydroxide was then added to each flask and hydrolysis was allowed to proceed for 90 minutes.

The hydrolysates were cooled, a drop of 1 per cent alcoholic thymolphthalein was added and neutralization was accomplished by the addition of glacial acetic acid to the disappearance of the blue color. In a few assays, chiefly those on hays and milk products, the solu-

tion color masked the end point of thymolphthalein so that it was necessary to neutralize to a pH of .8-9 with the aid of alkacid test paper as an indicator.

The neutralized solutions were suction filtered into 125 ml. flasks through No. 0 Buchner funnels, fitted with No. 2 filter paper overlaid with an asbestos mat. The boiling flasks were washed with six 2 ml. portions of distilled water, each washing in turn being used to wash the filter mat. The final volume of filtrate and washings was 35-40 milliliters.

Six milliliters of a 2 per cent methanol solution of Reinecke salt* was added to the combined filtrate and washings in the suction flasks. The flasks were placed in a refrigerator at 5°C. for at least 2 hours to assure complete precipitation of the choline as choline reineckate.

The precipitated choline reineckate was separated by suction filtering through 30 ml. fritted

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*Reinecke salt was made in the laboratory according to the method of Block and Bolling (18). Fresh methanol solutions were prepared at least once weekly. Both the solution and the dry salt were stored in a refrigerator.

In the early stages of the study an attempt was made to use Reinecke salt which was available commercially, but this was found to be unsatisfactory and it was necessary to discard all the results obtained.

glass funnels of medium porosity using 500 ml. suction flasks. The precipitation flasks were washed clear of precipitate with three 2.5 ml. portions of n-propanol, which in turn were used to wash the funnels. Suction was continued until the precipitates were dry.

The dried pink-colored precipitate* of choline reineckate on the filter disk was taken into solution by the addition of several portions of acetone. The acetone solution (total volume not over 20 ml.) was drawn off by gentle suction into a 125 ml. suction flask.

The acetone solution of choline reineckate was then quantitatively transferred to a 25 ml. glass stoppered volumetric flask. The flask was made up to volume and shaken. The concentration of choline reineckate in this solution was determined by means of an Evelyn Photoelectric Colorimeter, using filter No. 515.

The final result, expressed in milligrams of choline chloride, was obtained by reference to a standard curve, Figure I, constructed from values obtained for solutions of known choline chloride concentration.

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*In almost all cases variable amounts of other materials precipitated out when the Reinecke salt was added. This did not interfere with the determination since the extraneous precipitate did not go into solution when the choline reineckate was dissolved with acetone. However, the precipitate, especially that from milk products, and to a lesser extent that from grasses, markedly reduced the rate of filtration, and it was found necessary to overlay the filter disc with glass wool to facilitate filtration.

Preparation of a Standard Curve.

To prepare solutions of known choline chloride concentration, approximately 100 milligrams of choline chloride in a tared weighing bottle was dried in an oven at 130°C for two hours. After the dry weight of choline chloride had been determined, it was taken into solution in absolute methanol. Final dilution was made in a 100 ml. volumetric flask, so that the concentration of choline chloride could be accurately calculated.

Aliquots, measured with Normax pipettes, of 2, 3, 4, 5, 6, 7 and 8 milliliters of this solution were carried through the determination as described under "Assay Procedure". The colorimeter readings and "L values" obtained for known amounts of choline chloride are shown in Table 2.

A standard curve, Fig. I, page 16, was constructed from the values given in Table 2. The standard curve used in practice was drawn to a large scale so that the "L values" obtained from readings on the assay material could be plotted on the abscissa and the choline chloride equivalents read quite accurately from the ordinate.

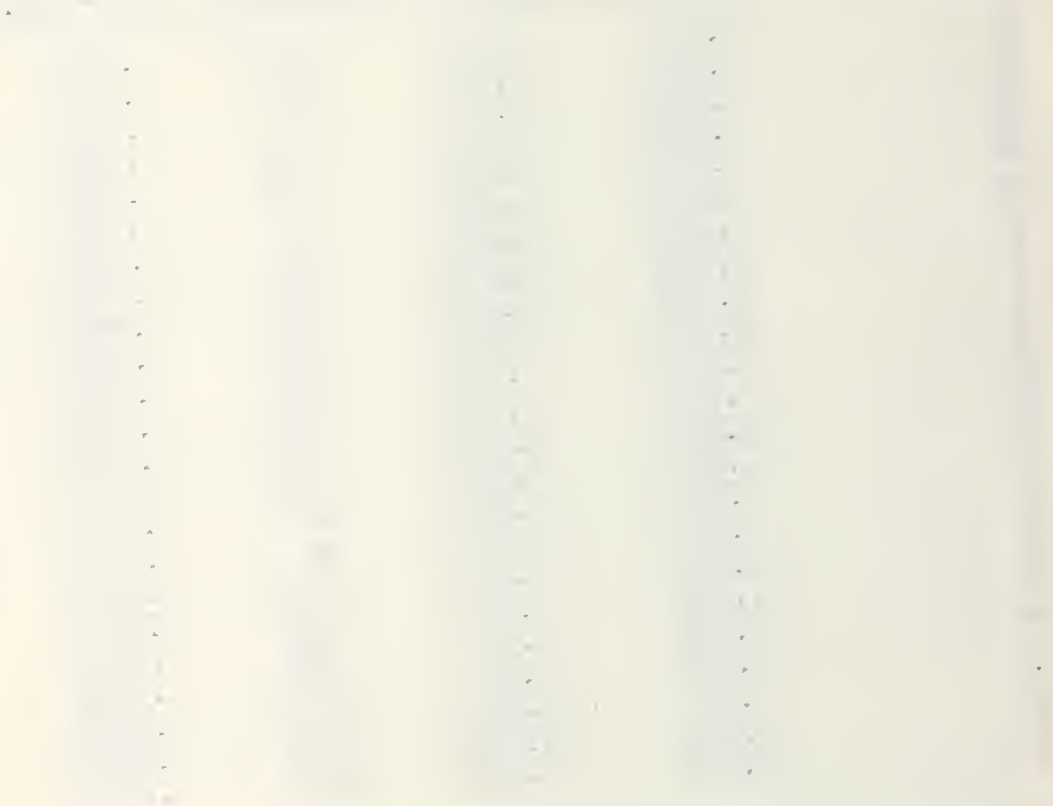
Standards were run periodically, but there was so little variation in the readings obtained that it was not necessary to alter the standard curve during the five month period in which most of the assays were done. Periodic recovery trials indicated a recovery of from 97 to 102 per cent of choline chloride added to assay samples.

Table 2.

Colorimeter and "L Values" Corresponding to Given Weights of Choline Chloride.

Ml. of Solution Used	Concentration Choline Chloride* mg./ml.	Choline Chloride mg.	Photoelectric Colorimeter Reading	L Value L=2-log. G
2	1.265	2.530	74^{2}	.1278
3	1.158	3.474	66^{3}	.1756
3	1.158	3.474	66^{1}	.1788
3	1.158	3.474	66	.1805
3	1.265	3.795	63^{3}	.1956
4	1.158	4.632	58^{1}	.2347
4	1.158	4.632	58	.2366
4	1.158	4.632	57^{2}	.2403
4	1.265	5.060	54^{3}	.2616
5	1.158	5.790	50^{2}	.2970
5	1.158	5.790	50	.3010
5	1.158	5.790	49^{2}	.3050
5	1.265	6.325	47^{2}	.3230
5	1.265	6.325	47^{1}	.3250
5	1.316	6.580	46^{1}	.3350
5	1.316	6.580	46	.3370
6	1.158	6.948	44	.3570
6	1.158	6.948	43^{2}	.3620
6	1.265	7.590	40^{3}	.3900
7	1.158	8.106	38^{3}	.4120
7	1.158	8.106	37^{3}	.4230
7	1.265	8.855	35^{1}	.4530
8	1.158	9.264	33^{2}	.4750

* Variations in concentration of choline chloride are accounted for by the fact that several preparations were used in an effort to compensate for errors that might arise from weighing and dilution.



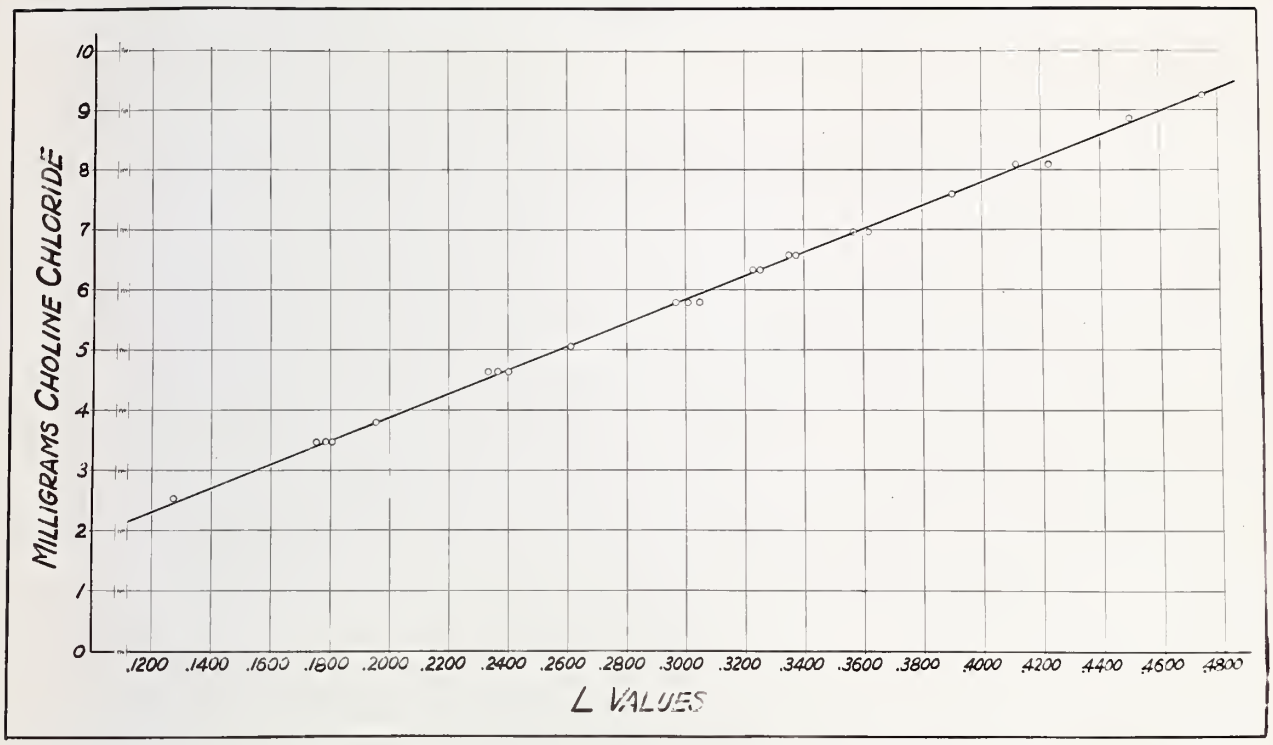


Fig. 1. "L Values" obtained with known quantities of choline chloride.

RESULTS AND COMMENTS.

The results of choline assays on a number of grains, hays and by-products are summarized in Table 3. The individual assay results used in compiling this table are shown in Appendix Tables II to IX.

Table 3.

Choline Content of Grains, Hays and By-Products Used in Feeds.

Section	Product	No. of Samples	Protein Range %	Choline Chloride Range mg./g.	Choline Chloride Average mg./g.
I	Grain:				
	High Protein Wheat	9	19.8-14.2	0.77-1.03	0.88
	Med. Protein Wheat	8	13.7-12.4	0.78-1.02	0.86
	Low Protein Wheat	6	10.7-8.6	0.82-0.93	0.89
	High Protein Oats	6	16.4-13.1	0.97-1.20	1.05
	Medium Protein Oats	11	12.7-10.8	0.87-1.02	0.98
	Low Protein Oats	8	9.6-8.2	0.89-1.12	1.00
	High Protein Barley	4	18.4-16.3	1.13-1.32	1.20
	Medium Protein Barley	3	13.7-12.9	1.03-1.20	1.15
	Low Protein Barley	4	10.6-9.1	0.95-1.14	1.05
	Yellow Corn	1	-	-	0.43
	Flaxseed	2	-	1.04-1.13	1.08
II	Legume Hays:				
	Alfalfa	6	-	1.07-1.98	1.51
	Altaswede Clover	1	-	-	1.36
	Sweet Clover	1	-	-	1.11

Table 3. (Continued)

Section	Product	No. of Samples	Protein Range %	Choline Chloride Range Mg./g.	Choline Chloride Average mg./g.
III	Grass Hays, Oat Straw & Silage:				
	Timothy	1	-	-	0.73
	Brome	4	-	0.33-0.66	0.43
	Crested Wheat Grass	1	-	-	0.54
	Oat Straw	1	-	-	0.21
	Oat Silage	1	-	-	0.88
IV	Plant By-Products:				
	Bran	2	-	1.43-1.48	1.46
	Shorts	2	-	1.54-1.58	1.56
	Middlings	2	-	1.20-1.44	1.32
	Feeding Wheat Germ	3	-	2.56-2.60	2.58
	Pure Wheat Germ	1	-	-	3.25
	Linseed Oil Meal	4	-	1.29-1.68	1.49
	Soy Bean Meal	5	-	2.70-3.15	2.49
V	Animal By-Products:				
	Meat Meal	6	-	1.83-2.88	2.42
	Tankage	2	-	1.48-1.70	1.59
	Blood Meal	2	-	0.43-0.91	0.68
	Cracklings	1	-	-	2.19
	Fish Meal	9	-	3.42-5.23	4.39
VI	Milk Products:				
	Skimmilk powder	7	-	1.56-1.86	1.70
	Buttermilk powder	8	-	2.09-2.59	2.31
	Whey powder	3	-	2.13-2.36	2.23
VII	Misc.:				
	Brewer's Yeast	1	-	-	3.56

Table 3. (Continued)

Section	Product	No. of Samples	Protein Range %	Choline Chloride Range mg./g.	Choline Chloride Average mg./g.
VIII	Mixed Poultry Feeds:				
	Chick Starter	1	-	-	1.39
	Turkey Starter	2	-	1.42-1.82	1.62
	Hatching Mash	1	-	-	1.49

Table 3 is divided into sections on a basis of similarity of nature or origin of feeds.

From section I it may be noted that:

1) There is comparatively little difference in the choline content of the three cereal grains, although there is a suggestion that they might be rated barley→oats→ wheat as sources of choline.
2) There appears to be no correlation between the protein and choline content of grains.
3) The single sample of yellow corn assayed contained only about one half as much choline as the cereal grains. Engel (16) shows a similar value for a single sample.
4) Flaxseed is about equal to the cereal grains in choline content.

From sections II and III of the table it may be noted that:

1) The legume hays are much better sources of choline than the grass hays.
2) Oat straw is very deficient in choline.
3) The choline content of hays is highly variable, not only as between different kinds of hay, but as between different samples of the same kind.

From section IV it may be noted that:

1) Plant by-products tend to be richer in choline than the whole seed from which they are derived.
2) Pure wheat germ is rich in choline, while feeding wheat germ which is diluted with bran is intermediate between pure wheat germ and bran in choline content.

From section V it may be noted that:

1) Fish meal is an excellent source of choline.
2) Blood meal is comparatively deficient in choline.
3) Meat meal is superior to tankage as a source of choline.

From section VI it may be noted that:

1) Skimmilk, buttermilk and whey powders are reasonably good sources of choline.
2) Buttermilk and whey powders contain from 30 to 35% more choline than does skimmilk powder.

In view of the recognized importance of choline in poultry rations, it is regrettable that time was not available for the assay of more mixed poultry feeds than are listed in section VIII. The following comments may be made regarding the results listed....

1) Ewing (19) suggests that chick starters should contain approximately 1.5 mg. of choline per gram. It would therefore appear that the chick starter assayed was marginal in this factor.

2) The recommended (20) level for choline in turkey starters is 2.0 mg. per gram. On this basis one of the turkey starters assayed was deficient in choline, while the other was marginal in choline content. It is known that the starter which assayed 1.82 mg. of choline chloride per gram was fortified with choline chloride.

Summary of Table 3.

In the following table six broad classes of feeds are listed in order of ascending choline content on the basis of the assay results shown in Table 3. The average value shown for the cereal grains is a true average of the results obtained on the 59 samples of cereal grain assayed. The average value shown for the other classes is an average of the average figures shown in the last column of the corresponding section in Table 3.

Table 4.

Choline Content of Different Classes of Feeds.

Class	Av. Choline mg./g.
Grass hays	0.58
Cereal grains	1.01
Legume hays	1.33
Plant by-products	2.10
Milk products	2.12
Animal by-products	2.65*

* The values for blood meal are omitted.

In addition to the assays reported above, a number of choline determinations were done on mill fractions of three samples of wheat which had been milled experimentally.* The object of conducting such assays was to study the possibility of differences in distribution of choline in various fractions of wheats of different protein content. The assay results are shown in Table 5.

Table 5.

Choline Content of Mill Fractions of Three Samples of Wheat Selected for Assay on the Basis of Difference in Protein Content.

	Choline Chloride		
Sample	5-W-1 Protein 19.8% mg./g.	5-W-2 Protein 13.7% mg./g.	5-W-3 Protein 9.1% mg./g.
Flour	0.89	0.70	0.83
Bran	1.40	1.18	1.05
Shorts	1.56	1.33	1.32
Feed Flour	1.44	1.16	1.17
Germ (diluted with bran)	1.47	1.34	1.40
Whole Grain, by assay	1.03	0.85	0.91
Whole Grain, by calculation from values obtained on fractions	1.08	0.83	0.95

Comments on Table 5.

1) The results do not indicate any correlation between the protein and choline content of wheat.

2) No correlation is shown between the protein content of wheat and the distribution of choline in various fractions of the kernel.

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* The mill fractions were supplied through the courtesy of the Department of Plant Science, University of Alberta.

3) The lowest concentration of choline is found in the flour.

4) Agreement is good between the choline values obtained by assay of the whole grain and those obtained by calculating from assay values for the different fractions.

GENERAL DISCUSSION.

In general, classes of feed which are rich in protein are relatively rich in choline, and those which are low in protein are deficient in choline. This trend is illustrated in Table 4. and might be expected, since it is believed that most of the choline in plant and animal products occurs as lecithin and lecithin is generally associated with protein.

In the case of hays, this trend is apparent within a given class of feedstuffs. For example, alfalfa hays, which are generally richer than grass hays in protein, are also richer in choline. A further illustration will be found in Appendix Table V, where it is shown that, for any one kind of hay, samples which were green and of good quality contained approximately twice as much choline as did ripe or weathered samples.

A trend towards increasing choline content with increasing protein content is not apparent in the grains, and no complete explanation can be offered for this apparent exception. A partial explanation as suggested by Engel (16) may be that in plants choline is more concentrated in actively growing portions than in ripe storage portions. Variations in the choline content of the ripe kernel might therefore be expected to be small, particularly in view of the fact that the germ, which contains the greatest concen-

tration of choline and is the potentially active portion of the seed, constitutes a relatively small proportion of the kernel.

Linseed oil meal was found to be superior to flaxseed as a source of choline. This extends and confirms the results obtained by Engel (16) on cottonseed, peanut and soybean meal to indicate that the meals resulting from the extraction of oil from oil-bearing seeds are richer in choline than are the whole seeds.

Apparently little choline is removed from seeds with the removal of the oil. In explanation, Engel suggests that most of the choline in the seeds occurs in phospholipids which are held in a protein complex.

Milk products showed relatively little variation in choline between individual samples. The choline content of buttermilk powder and whey powder was found to be about a third greater than that of skimmilk powder. Buttermilk powder is essentially the dried skimmilk residue from the churning process. However, during churning the enveloping lecithin layer of the fat globules becomes destabilized and is freed to the residue. At least part of the increased choline content of buttermilk powder over skimmilk powder can be attributed to this destabilized lecithin.

Whey powder, which is the dried residue from cheese manufacture, is essentially the solids left after the casein and most of the fat are removed from milk

by coagulation. Its high choline content is probably partially due to the fact that some lecithin is destabilized during coagulation of the casein and remains in the whey, possibly in association with the lactalbumin. In addition, the total solids content of whey is much lower than that of buttermilk or skimmilk and therefore the choline is more concentrated during the drying process. At least part of the choline content of all three powders arises from the lecithin layer on the minute fat globules which remain in the final product.

Of all the feed samples assayed, fish meal was found to be the best source of choline, but reference to Appendix Table VII will show that variation between individual samples was high. Possibly this variation may be due to differences in the species of fish used or to the use of varying proportions of whole fish and trimmings in the preparation of the meal. Processing methods may also affect the choline content of the product.

In contrast to the findings of Engel (16) meat meal was found to be richer than tankage in choline. Blood meal was found to be relatively low in choline. Since appreciable amounts of blood meal may be added to tankage, but not to meat meal, this may partially explain the difference found between meat meal and tankage.

Since the grains which constitute a relatively large proportion of poultry rations are relatively poor sources of choline, and since the choline requirement

of birds is known to be relatively high, it would appear that serious consideration should be given to the possible need of choline chloride supplementation in mixed poultry feeds. The limited number of mixed poultry rations which were assayed were either marginal or deficient in choline.

SUMMARY.

1) Choline analyses were conducted on a number of grains, hays and by-products commonly used in livestock and poultry feeds.

2) In general the choline content of feeds tends to vary directly with the protein content.

3) The germ and outer coats of cereal grains are better sources of choline than is the whole grain.

4) Seed oil meals are richer in choline than are the whole seeds from which they are prepared.

5) Legume hays are slightly better than grains as a source of choline.

6) Grass hays, especially those made from mature grass, contain relatively little choline.

7) By-products of animal origin are, in general, relatively rich in choline although blood meal was found to be a comparatively poor source of this factor.

8) The possibility of a need for choline supplements in poultry rations, especially in turkey rations, is suggested.

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APPENDIX TABLES.

APPENDIX

Table I.

Approximate Sample Size and Pumice Used for Different Products Analysed.

Sample	Sample Size (grams)	Approximate Pumice Used (grams)
Wheat	5-7	8
Oats	5	7
Barley	5	7
Flour	5	10
Bran	4	0
Shorts	4	0
Middlings	4	7
Wheat Germ	2	0
Corn	8	0
Flax	5	7
Linseed Oil Meal	3	7
Soy Bean Oil Meal	2	7
Meat Meal	3	5
Blood Meal	3	5
Tankage	3	5
Fish Meal	1 1/2	5
Brewer's Yeast	2	10
Mixed Feeds	4	5
Milk Powder	4	10

Table II.

Description and Choline Content of Wheat Samples Assayed.

Sample No.	Station	Variety	Soil Type	Protein %	Choline Chloride mg./g.
High Protein.					
5-W-1	Conrad	--	Brown	19.8	1.03
4-W-1	Conrad	--	Brown	17.3	0.88
4-W-13	Acadia Valley	Marquis	Brown	15.4	0.85
4-W-3	Airdrie	Marquis	Sh.Black	15.3	0.84
4-W-16	Drumheller	Marquis	Dk.Brown	15.3	0.82
4-W-5	Consort	Marquis	Brown	14.4	0.94
4-W-11	Edmonton	Marquis	Black	14.4	0.87
4-W-19	Pincher Creek	Red Bobs	Sh.Black	14.2	0.89
4-W-17	Lomond	Marquis	Brown	14.2	0.77
Medium Protein.					
4-W-18	Nobleford	Marquis	Dk.Brown	13.7	0.78
5-W-2	Bremner	--	Black	13.7	0.85
4-W-20	Baldonnel	Marquis	Gray	13.6	0.84
4-W-4	Castor	Marquis	Dk.Brown	13.5	0.84
4-W-7	St. Paul	Marquis	Black	13.2	1.02
4-W-15	Craigmyle	Marquis	Dk.Brown	13.1	0.82
4-W-6	Stettler	Marquis	Sh.Black	12.6	0.93
4-W-10	Bon Accord	Marquis	Black	12.4	0.85
Low Protein.					
4-W-12	Fallis	Marquis	Gray	10.7	0.93
4-W-14	Claresholm	Red Bobs	Dk.Brown	10.6	0.87
4-W-8	Winfield	Marquis	Gray	10.6	0.87
5-W-3	Stony Plain	--	Black	9.1	0.91
4-W-2	Fallis	Red Bobs	Gray	8.9	0.82
4-W-9	Athabasca	Marquis	Gr.Trans.	8.6	0.93

Table III.

Description and Choline Content of Oat Samples Assayed.

Sample No.	Station	Variety	Soil Type	Protein %	Choline Chloride mg./g.
High Protein.					
5-0-1	Taber	--	Brown	16.4	1.20
4-0-1	Wrentham	Victory	Dk.Brown	15.9	0.97
4-0-19	Drumheller	Eagle	Dk.Brown	13.7	1.09
4-0-22	Pincher Creek	Victory	Sh.Black	13.6	0.96
4-0-6	Chauvin	Victory	Dk.Brown	13.4	1.03
4-0-12	St. Paul	Victory	Black	13.1	1.02
Medium Protein.					
4-0-2	Edmonton	Victory	Black	12.7	1.15
4-0-5	Castor	Victory	Dk.Brown	12.3	1.00
5-0-2	Clover Bar	--	Black	12.3	0.97
4-0-4	Airdrie	Victory	Sh.Black	12.2	0.97
4-0-10	Fallis	Victory	Gray	11.9	1.02
4-0-21	Nobleford	Victory	Dk.Brown	11.6	0.97
4-0-8	Consort	Victory	Brown	11.5	0.87
4-0-16	Edmonton	Victory	Black	11.4	1.02
4-0-18	Craigmyle	Victory	Dk.Brown	11.2	0.88
4-0-11	Stettler	Victory	Sh.Black	11.0	0.96
4-0-9	Dickson	Victory	Bl.Trans.	10.8	1.02
Low Protein.					
5-0-3	Warburg	--	Gray	9.6	1.12
4-0-14	Bon Accord	Victory	Black	9.5	0.94
4-0-20	Lomond	Victory	Brown	9.5	1.06
4-0-15	Fallis	Victory	Gray	9.3	0.95
4-0-17	Claresholm	Victory	Dk.Brown	8.9	1.06
4-0-13	Winfield	Victory	Gray	8.8	0.94
4-0-7	Cheddarville	Victory	Gray	8.2	0.89
4-0-3	Bon Accord	Victory	Gr.Trans.	8.2	1.02

Table IV.

Description and Choline Content of Barley Samples Assayed.

Sample No.	Station	Variety	Soil Type	Protein %	Choline Chloride mg./g.
High Protein.					
5-B-11	Purple Springs	--	Brown	18.4	1.13
5-B-6	Legend	--	Brown	18.2	1.19
5-B-7	Skiff	--	Brown	17.6	1.16
5-B-2	Wrentham	--	Dk.Brown	16.3	1.32
Medium Protein.					
5-B-28	Thorsby	--	Bl.Trans.	13.7	1.03
5-B-17	Burdett	--	Brown	13.6	1.20
5-B-13	Purple Springs	--	Brown	13.6	1.17
5-B-23	Rimbey	--	Bl.Trans.	12.9	1.20
Low Protein.					
5-B-44	Spruce Grove	--	Black	10.6	1.14
5-B-18	Eckville	Olli	Gray	9.2	0.95
5-B-24	Rimbey	--	Bl.Trans.	9.1	1.07

Table V.

Description and Choline Content of Roughages Assayed.

Sample No.	Product	Station	Notes on Quality	Choline Chloride mg./g.
Legume Hays.				
4-G-8	Alfalfa	Lacombe	Leafy green	1.98
4-G-12	Alfalfa	Metiskow	Very ripe	1.42
4-G-10	Alfalfa	Lethbridge	Fair	1.44
4-G-14	Alfalfa	Manyberries	Good	1.77
4-G-15	Alfalfa	Edmonton	Good	1.38
4-G-16	Alfalfa	--	Weathered, ripe	1.07
4-G-4	Altaswede Clover	Beaverlodge	Good	1.36
4-G-5	Sweet Clover	Beaverlodge	Good	1.11
Grass Hays.				
4-G-6	Crested Wheat Grass	Beaverlodge	Good	0.54
4-G-7	Brome Grass	Medicine Hat	Very ripe	0.33
4-G-13	Brome Grass	Manyberries	Very ripe	0.39
4-G-9	Brome Grass	Lacombe	Good	0.66
4-G-11	Brome Grass	Lethbridge	Good	0.56
5-G-18	Timothy	Bon Accord	Very good	0.73
Straws and Silage.				
5-G-19	Oat Straw	Bon Accord	Ripe oats	0.21
5-G-20	Oat Silage	Edmonton	Good	0.88

Table VI.

Choline Content of Milk Products Assayed.

Sample No.	Product	Approx. Date of Collection	Choline Chloride mg./g.
S-1	Skimmilk powder	May, 1945	1.71
S-2	Skimmilk powder	May, 1945	1.67
S-3	Skimmilk powder	May, 1945	1.75
S-12	Skimmilk powder	May, 1945	1.56
S-23	Skimmilk powder	May, 1945	1.86
S-29	Skimmilk powder	June, 1945	1.71
S-30	Skimmilk powder	February, 1946	1.62
S-5	Buttermilk powder	May, 1945	2.55
S-6	Buttermilk powder	May, 1945	2.53
S-7	Buttermilk powder	May, 1945	2.39
S-17	Buttermilk powder	May, 1945	2.43
S-19	Buttermilk powder	May, 1945	2.09
S-26	Buttermilk powder	May, 1945	2.14
S-20	Buttermilk powder	May, 1945	2.19
S-28	Buttermilk powder	May, 1945	2.14
S-4	Whey powder	May, 1945	2.23
S-16	Whey powder	May, 1945	2.36
S-18	Whey powder	May, 1945	2.13

Table VII.

Choline Content of Animal By-Products Assayed.

Sample No.	Product	Notes	Choline Chloride mg./g.
S-8	Meat Meal	Dry render	2.18
S-14	Meat Meal	--	2.86
S-24	Meat Meal	50% Protein	1.83
S-31	Meat Meal	51% Protein	2.35
S-32	Meat Meal	50% Protein	2.77
S-33	Meat Meal	50% Protein	2.48
S-34	Tankage	Wet render	1.48
S-35	Tankage	Wet render	1.70
S-51	Blood Meal	Pure Blood	0.43
S-52	Blood Meal	Approx. 95% Blood	0.91
S-21	Crackling	May, 1945	2.19
S-9	Fish Meal	Protein 71%	5.23
S-10	Fish Meal	Protein 75%	4.92
S-13	Fish Meal	--	3.42
S-15	Fish Meal	--	5.05
S-16	Fish Meal	Protein 69%	4.80
S-22	Fish Meal	--	3.64
S-27	Fish Meal	--	4.56
S-36	Fish Meal	Protein 66%	3.85
S-37	Fish Meal	--	4.04

Table VIII.

Choline Content of Plant By-Products, Corn, Flax, Brewer's Yeast Samples.

Sample No.	Note	Choline Chloride mg./g.
Middlings		
S-48	16% Protein	1.20
S-49	--	1.44
Bran		
S-53	15% Protein	1.48
S-54	--	1.43
Shorts		
S-58	16% Protein	1.54
S-59	--	1.58
Feeding Wheat Germ		
S-63	All samples diluted	2.56
S -64	with bran	2.60
S-65		2.58
Wheat Germ		
S-76	Pure Germ	3.25
Linseed Oil Meal		
S-40	35% Protein	1.63
S-41	38% Protein	1.68
S-42	38% Protein	1.29
S-43	--	1.37
Soybean Oil Meal		
S-11	--	2.70
S-25	--	2.87
S-44	44% Protein	3.12
S-45	--	3.15
S-46	44% Protein	2.84
Flax		
S-38	February, 1946	1.04
S-39	February, 1946	1.13
Corn		
S-47	Ground Yellow Corn	0.43
Brewer's Yeast		
S-60	34.5% Protein	3.56

Table IX.

Choline Content of Several Commercial Poultry Feeds.

Sample No.	Protein %	Choline Chloride mg./g.
Commercial Chick Starter		
S-72	18% Protein	1.39
Commercial Hatching Mash		
S-74	18% Protein	1.49
Commercial Turkey Starter		
S-73	25% Protein	1.42
S-75	--	1.82